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Larry J. Sadler
Iowa State University, ljsadler@iastate.edu

C. D. Hagen
Value-Added Science and Technologies (VAST)

Chong Wang
Iowa State University, chwang@iastate.edu

T. M. Widowski
University of Guelph

Anna Johnson
Iowa State University, johnsona@iastate.edu

See next page for additional authors

Authors
Larry J. Sadler, C. D. Hagen, Chong Wang, T. M. Widowski, Anna Johnson, and Suzanne T. Millman

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Effects of flow rate and gas mixture on the welfare of weaned and neonate pigs during gas euthanasia[1]

L. J. Sadler*[2], C. D. Hagen[†], C. Wang[‡#], T.M. Widowski[§], A. K. Johnson , S. T. Millman*[‡]

*Department of Biomedical Sciences, Iowa State University, Ames, IA, 50011

[†]Value-Added Science and Technologies (VAST), Mason City, IA, 50401

[‡]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, 50011

[#]Department of Statistics, Iowa State University, Ames, IA, 50011

[§]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada

Department of Animal Science, Iowa State University, Ames, IA, 50011

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[2]Corresponding author: ljsadler@iastate.edu

[2]Current address: 2356 Kildee Hall, Department of Animal Science, Iowa State University, Ames, IA, 50011.

ABSTRACT: The objectives of this study were to assess efficacy and welfare implications of gas euthanasia when applied to weaned and neonate pigs. Parameters associated with welfare, which were measured prior to loss of consciousness, included open-mouth breathing, ataxia, righting response, and escape attempts. Two age groups (weaned and neonate) were assessed in 9 gas treatments arranged in a 2 × 4 factorial design, with 2 gas types (CO_2 = 100% CO_2 or 50:50 = 50:50 CO_2:argon) and 4 flow rates (box volume exchange/min: slow = 20%; medium = 35%; fast = 50%; or prefill = prefilled followed by 20%) and a control treatment in which ambient air was passed through the box. Pig pairs (10/treatment) were placed in a modified Euthanex AgPro system (Euthanex Corp., Palmer, PA). Behavioral and physiological responses were observed directly and from video recordings for latency, duration, prevalence (percent of pigs affected), and frequency (number of occurrences/pig). Data were analyzed as linear mixed models or with a Cox proportional hazard model as appropriate. Piglet pair was the experimental unit. For the weaned pig, welfare was superior with CO_2 relative to 50:50 within 1 or more flow rates based on reduced duration of open-mouth breathing, duration of ataxia, frequency of escape attempts, and duration and frequency of righting response ($P < 0.05$). No measured parameters indicated superior welfare with the use of 50:50, whereas latencies to loss of posture and last movement favored CO_2 ($P < 0.05$). Faster flow rates were associated with reduced ($P < 0.05$) duration or frequency of open-mouth breathing, ataxia, and righting response, as well as superior ($P < 0.05$) indicators of efficacy, including latencies to loss of posture, gasping and last movement, relative to slower flow rates. Weaned pigs were more likely to defecate ($P < 0.01$), display nasal discharge ($P < 0.05$), and display longer ($P < 0.001$) latencies to loss of posture and last movement than neonates. Duration of ataxia was the only parameter for which neonates were superior ($P < 0.01$) to weaned pigs during euthanasia. As such, a 50:50 CO_2:argon gas mixture and slower flow rates should be avoided when euthanizing weaned or neonate pigs with gas methods. Neonate pigs succumb to the effects of gas euthanasia quicker than weaned pigs and display fewer signs of distress.

Key words: animal welfare, argon, carbon dioxide, gas euthanasia, piglet, swine

INTRODUCTION

Millions of weaned and suckling pigs are euthanized annually in the United States (Sadler, 2013). Swine producers and veterinarians generally agree that euthanasia is appropriate when chances of survival are low and there is suffering due to injury or illness. Euthanasia is comprised of 2 stages: 1) induction of unconsciousness (insensibility); and 2) death. It is the induction phase that is critical to ensure pig welfare. The entire process, including death, is important to ensure practical and timely implementation. Blunt-force trauma is currently the most common euthanasia method for pigs less than 5.4 kg, but is recognized as being psychologically difficult for some caretakers to perform (Morrow et al., 2010) and has been receiving criticism (Daniels, 2010). These factors have prompted the U.S. swine industry to develop and refine alternative euthanasia methods for the pig, in particular inhalant agents.

Carbon dioxide (CO_2) is the most commonly implemented gas for swine euthanasia (Daniels, 2010), but CO_2 is mildly acidic, which may cause irritation to mucus membranes (Danneman et al., 1997). This has led to questions about whether CO_2 is a humane option for pig anesthesia and euthanasia (Wright et al., 2009).

Argon (Ar), or CO_2:Ar mixtures, have been proposed as alternatives (Raj and Gregory, 1996), because Ar is a noble gas, and, as such, is unlikely to be reactive throughout the physiological systems (Mann et al., 1997). Excess latencies to loss of posture and respiratory arrest, along with low efficacy, have been observed in 90% Ar and 30:60 CO_2:Ar gas mixtures (Raj 1999), potentially limiting the practicality of implementation in research and production settings. There is little published research that addresses proper flow rates for gas euthanasia of neonates and weaned pigs. Therefore, the objectives of this research were to examine efficacy of CO_2 vs. a CO_2:Ar gas mixture administered at 4 flow rates during euthanasia and effects on weaned and neonate pig welfare.

MATERIALS AND METHODS

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee.

Experimental design

There were 2 age groups (weaned and neonate) of pigs and 9 gas treatments in a 2 × 4 factorial arrangement, with 2 gas types (CO_2 [100% CO_2] vs. 50:50 [50% CO_2:50% Ar]) and 4 flow rates (box volume exchange/min: slow [20%], medium [35%], fast [50%], or prefilled followed by 20%). The flow rates for 100% CO_2 were 2.26, 1.59, and 0.91 m^3/h, respectively, and 1.13:1.13, 0.79:0.79, and 0.45:0.45 m^3/h for the 50:50 gas mixture, respectively. For the prefilled treatment, prior to pig placement, gas was provided at 50% box volume/min for a minimum of 5 min. Additionally, a control treatment was included in which ambient (AMB) air was passed through the box. Pig pairs (10/treatment), consisting of male-female matched penmates (weaned) or littermates (neonate), were used to reduce isolation and social distress. Order of treatments was assigned randomly prior to the day of treatment, with one replication of all 9 treatments conducted on a given day. The first pig pair selected was assigned to the first treatment to be run, proceeding in this fashion sequentially until all treatments were filled.

Animals and housing

The experiment was conducted from May through September 2010, with a total of 340 pigs. Weaned pigs (90 female-male pairs) ranged from 16 to 24 d of age, weighed 4.8 ± 0.2 kg, and originated from a commercial genetic line (PIC, Hendersonville, TN) sourced from the Iowa State University Swine Nutrition Farm. Neonates (80 female-male pairs) were 1.4 ± 0.1 d of age, 2.6 ± 0.1 kg, and were classified as suckling pigs less than 3 d of age. These pigs were housed and sourced from either the Iowa State University Teaching Farm or a commercial swine farm located in western Iowa. Pigs from the Iowa State University Teaching Farm were a composite of Duroc, Landrace, Yorkshire, and Hampshire (9 female-male pairs), whereas pigs from the commercial farm were from a mating of Landrace × Yorkshire females to Duroc performance-line sires (71 female-male pairs).

Euthanasia equipment

Gases were administered to the pigs via a Euthanex AgPro system. This gas delivery apparatus was designed by Euthanex Corporation (Palmer, PA), a manufacturer of gas delivery systems for rodents and small animals. The system allows for variable and precise administration of gas types, mixtures, flow rates, and delivery times. To facilitate behavioral observation, the box was constructed of clear plastic on the top and front panels. The remaining 4 panels were constructed of opaque plastic. Inside dimensions of the box were 43 cm (wide) × 60 cm (long) × 30 cm (high) resulting in a total volume of 76,4556 cm^3. The box had two 0.64-cm-diameter inlet valves located at 12.70 cm and 22.86 cm from the side (for CO_2 and Ar, respectively) and 3.81 cm from the top, and a 0.95-cm outlet valve was located on the opposite panel from the inlet valves, 30.48 cm from the side and 6.35 cm from top. The gas flowed through 3.25 m of 0.64-cm-diameter hoses prior to entering the box. The floor was fitted with a rubber floor mat (Kraco, Enterprises, LLC, Compton, CA) for traction. The CO_2 gas used was industrial grade (99% pure; Iowa State University Chemistry Store, Ames, IA), whereas the Ar had a guaranteed analysis of 99.99% pure (Iowa State University Chemistry Store, Ames, IA). Constant and precise gas flows were provided using compressed gas cylinders equipped with compressed gas regulators and meters (Western Enterprises, Westlake, OH). Prior to each treatment, the box was cleaned out using pressurized air from an air compressor and disinfected with Roccal (Pfizer Animal Health, New York, NY).

Enrollment and euthanasia procedure

Pig pairs were identified and marked with an animal-safe marker (LA-CO Industry, Elk Grove, IL). Pigs were then removed from their home pens and carried to the testing room. The testing room provided isolation, thereby minimizing noise and distractions. The room provided adequate ventilation ensuring escaped gases were not a concern to human safety. To habituate pigs to the euthanasia box, the pig pair was placed in the box for 10 min and then taken back to the home pen. A minimum of 1 h elapsed before the pair was placed back into the box. Upon placement, gas was immediately started and applied for 10 min. For gas treatments, pigs remained in the box until 10 min after last movement of both pigs was observed. Pigs were then removed and tested for insensibility and death. For the AMB treatment, pigs were removed from the box after 10 min and blunt-force trauma was applied in accordance with the American Association of Swine Veterinarians guidelines (NPB, 2009).

For ethical reasons, pigs that displayed movement following 10 min of exposure to the gas were removed from the box and checked for insensibility. Pigs that displayed signs of sensibility were immediately euthanized using blunt-force trauma. Pigs that were insensible were returned to the box and the euthanasia process, as described previously, repeated. This modification was sufficient to induce cessation of movement (involuntary) and heartbeat in all pigs.

Confirmation of insensibility and death

Each pig was removed individually from the box and was immediately checked for signs of insensibility (Whelan and Flecknell, 1992; Kissin, 2000; Grandin, 2010). Three tests were conducted: 1) corneal reflex response, in which the eye was touched with the tip of a finger for absence of an eye blink or withdrawal response; 2) pupillary reflex, in which a light-beam (Mini MAGLite, Mag Instrument, Inc., Ontario, CA) was shone into the eye and pupil observed for absence of constriction; and 3) nose prick, where a 20-gauge needle was touched to the snout distal to the rostral bone for absence of a withdrawal response. After insensibility was confirmed, auscultation was used to confirm absence of heartbeat.

Modification of study design

At the individual pig level, 75% of the weaned pigs did not achieve last movement during the initial 10 min of gas application of the slow flow rate 50:50 gas treatment. Of these, 47% of pigs were still sensible and blunt-force trauma was immediately applied. Another 53% were insensible, but maintained a heartbeat. These pigs were placed back in the box for up to an additional 10 min during which all achieved last movement. Due to ethical concerns regarding the high number of pigs requiring a secondary euthanasia step, the 50:50 slow treatment was not examined in the neonates, creating an unbalanced study design for this age group.

Environmental conditions

A HOBO data logger (U23-001; Onset Computer Corp, Bourne, MA) was placed within the box to record temperature (°C) and relative humidity (%), and was set to record every 10 s. Data were collected continuously throughout the treatment day and exported into a Microsoft Excel spreadsheet (version 2007; Microsoft, Redmond, WA). For each pig pair, environmental data were extracted for 3 time periods (entry into the box, loss of posture, and exit from the box).

Temperature within the box was relatively constant when gas was flowing, regardless of treatment (Table 1 and 2). Relative humidity showed slightly greater variation between treatments, with the average relative humidity between treatments ranging less than 8%. For weaned pig trials, mean starting temperature and relative humidity for all treatments was 26.24 °C and 68.35%, respectively; however, the neonate pig trials were conducted at a slightly lower temperature and relative humidity (23.1°C and 55.4%, respectively). Environmental differences likely resulted from procedures conducted on different farms and days. Temperature and relative humidity within the box changed little from when pigs were placed into the box until loss of posture. The average temperature change in the box, over all treatments, was only -0.16 °C, with the greatest average change within a single treatment of -0.35 °C in the 50:50 prefill treatment.

Relative humidity also changed little during this time, increasing 3.91% over all treatments, with the greatest change (5.43%) occurring in the weaned pigs at the fast flow rate.

Behavioral observations

Behavioral data were collected directly and via video recording. For direct observation, a single observer sat approximately 1.5 m from the box and recorded behavioral indicators of distress and consciousness (Table 3). Latency to last movement for the AMB treatment was determined from the time blunt-force trauma was applied, whereas latency for all other behaviors was determined from the point when each pig was placed into the box.

Video was captured using a Noldus Portable Lab (Noldus Information Technol., Wageningen, the Netherlands). Two color Panasonic cameras (WV-CP484; Panasonic Corp., Kadoma, Japan) were fed into a multiplexer, which allowed the image to be recorded onto a PC using HandiAvi (version 4.3; Anderson's AZcendant Software, Tempe, AZ) at 30 frames/s. Behavioral data were collected by 2 trained observers, blinded to treatments, using Observer (version 10.1.548; Noldus Information Technol., Wageningen, the Netherlands). Pigs were scored individually for behavioral and physiological indicators of distress and efficacy of the euthanasia process (Table 3). Prior to data collection, observers were trained to the ethogram, and scoring was not started until inter-observer reliability $k > 0.90$ was achieved. Inter- and intra-observer reliability were checked at the end of the observation period, with both having $k > 0.90$. Treatments were balanced between observers.

Statistical Analysis

Scored behaviors were assessed as latency, duration, percentage of pigs (analyzed as number of pigs displaying), or frequency of occurrence as appropriate for the parameter (Table 3). Data were analyzed using linear mixed models fitted with the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) or with a Cox proportional hazard model fitted with the PHREG procedure of SAS. Piglet pair served as the experimental unit. Least square means for each treatment group and the corresponding SE, or SEM, are reported. The linear model included the fixed effect of gas type (CO_2 or 50:50), flow rate (slow, medium, fast, or prefill), age (weaned or neonate), and all 2- and 3-way interactions. A random blocking effect of litter or pen was included in the statistical model, and the Kenward-Rogers method was utilized for determining the denominator d.f.. Statistical significance was established at $P \leq 0.05$ using a Sidak correction for multiple comparisons, unless otherwise noted. Sex, weight, and age (neonate pigs only) of the individual pig was examined, but had no effect ($P > 0.10$) on any results; thus, all were removed from all final models.

RESULTS

Weaned pigs

When the pigs were conscious (prior to loss of posture), duration of standing and locomotion did not ($P = 0.11$) differ between CO_2 and 50:50 or between flow rates (Table 4). Durations of oral and nasal behaviors, as well as licking and chewing, were shorter ($P < 0.05$) for prefill CO_2 and prefill 50:50 than other flow rates, and AMB produced longer ($P < 0.001$) durations of oral and nasal and licking and chewing behaviors when compared to all gas treatments. Escape attempts were only observed by weaned pigs subjected to 50:50 gas euthanasia treatments, and was performed by 10% of the pigs in the fast flow rate and 15% in all other flow rates (Table 5). However, there were no differences among gas types, flow rates, and AMB in the percentages of weaned pigs displaying defecation ($P = 0.24$), urination ($P = 0.36$), salivation ($P = 0.21$), or nasal discharge ($P = 0.54$; Table 5). It should be noted that ocular-orbit discharge was exhibited by 1 pig subjected to 50:50 slow and 1 pig in the CO_2 prefill treatment, but blood was never visibly present in discharges, and no weaned pigs were observed vomiting.

Within CO_2, latency to open-mouth breathing was least for prefill and longest for slow, and did not differ between fast and medium, whereas, among 50:50-euthanized pigs, latency to open-mouth breathing was shortest ($P < 0.05$) for prefill, and shorter ($P < 0.05$) for fast than medium and slow flow rates (Table 6). Moreover, latency to open-mouth breathing was much quicker ($P < 0.05$) for CO_2 than 50:50 when applied at the slow and medium flow rates. The proportion of weaned pigs displaying open-mouth breathing did not, however, differ between gas type ($P = 0.98$) or among flow rates ($P = 0.93$), with 80 to 100% of pigs displaying this behavior, and no pigs in the AMB displayed open-mouth breathing ($P < 0.001$; Table 5). Duration of open-mouth breathing increased ($P < 0.05$) as flow rates decreased within CO_2 euthanasia, and, among 50:50-euthanized pigs, durations of open-mouth breathing were greater ($P < 0.05$) in the medium and slow rates than either prefill or fast flow rates (Table 4). Furthermore, duration of open-mouth breading was grater ($P < 0.05$) for 50:50 than CO_2 at all flow rates.

Prior to loss of posture, 99% of pigs displayed ataxia. Regardless of flow rate, 50:50 caused longer ($P < 0.001$) durations of ataxia than CO_2 (Table 4). Among pigs euthanized with CO_2, the shortest ($P < 0.05$) duration of ataxia was observed in the prefilled box, the longest ($P < 0.05$) was observed in the slow flow rate, and medium caused longer ($P < 0.05$) induction of ataxia than the fast flow rate. Even though duration of ataxia was similar between the prefill and fast flow rates of 50:50, both reduced ($P < 0.05$) the duration of ataxia compared to the medium flow rate; slow rate of 50:50 induced the longest ($P < 0.05$) time in ataxia.

A righting response was observed in 10 to 60% of weaned pigs prior to loss of posture (Table 5). The number of righting attempt efforts by a single pig ranged from 0 to 12 (maximum attempts: 4, 1, 5, and 6 for CO_2 and 12, 5, 4, and 10 for 50:50 at prefill, fast, medium, and slow flow rates, respectively). Among the CO_2 euthanatized pigs, the duration of the righting response was shorter ($P < 0.05$) in prefill and fast relative to medium and slow; however, among 50:50-euthanzied pigs, shortest ($P < 0.05$) duration was observed in medium relative to prefill and slow, whereas the fast flow rate was shorter ($P < 0.05$) in duration than slow.

Within CO_2, prefill was the quickest ($P < 0.05$) to induce loss of posture, and took longer ($P < 0.05$) in slow than fast and medium flow rates (Table 6). Similarly, 50:50 prefill caused the shortest ($P < 0.05$) latency to loss of posture, whereas 50:50 at the slow caused the longest ($P < 0.05$) latency to loss of posture, and the time to loss of posture was less ($P < 0.05$) for fast than the medium flow rate. More specifically, loss of posture occurred faster ($P < 0.05$) in weaned pigs exposed to CO_2 than 50:50.

Within CO_2, muscle excitation was observed less ($P < 0.05$) frequently in slow compared to all other flow rates (Table 5), and, within 50:50, the prevalence of muscle excitation was less ($P < 0.05$) in prefill and slow relative to fast and medium. Furthermore, the prevalence of muscle excitation was less ($P < 0.05$) for 50:50 than CO_2, regardless of flow rate. Mean duration of muscle excitation was greater in CO_2 than 50:50 (< 7 s vs. < 4 s; results now shown). All pigs displayed clonic movements, with the exception of 1 pig in the prefill CO_2 gas treatment. Within CO_2, the slow flow rate was associated with longer ($P < 0.05$) duration of clonic movements than prefill, fast, and medium; however, although the duration of clonic movements was similar between prefill and slow in 50:50, the slow rate was associated with longer ($P < 0.05$) durations of clonic movements relative to fast and medium. Between the 2 gas types, differences in duration of clonic movements was observed only within the prefill flow rate.

Gasping was performed by 90 to 100% of the weaned pigs in CO_2 and 50:50, and there were no differences ($P = 0.86$) between gas types or flow rates; however, none ($P < 0.001$) of the pigs in the AMB treatment displayed this behavior (Table 5). Within CO_2, duration of gasping was longest ($P < 0.05$) for the slow relative to other flow rates, and gasping was longer ($P < 0.05$) in the prefill than the fast flow rate (Table 4). Similarly, gasping was longest ($P < 0.05$) at the slow rate in 50:50-euthanized pigs, and gasping was longer ($P < 0.05$) in prefill and medium than the fast flow rate.

Latency to last movement among CO_2-euthanized pigs was shorter ($P < 0.05$) in the prefill, fast, and medium flow rates than for the slow flow rate (Table 6). Latency to last movement was also longer ($P < 0.05$) with 50:50 than CO_2 gas, regardless of flow rate, and latency to last movement was quicker ($P < 0.05$) in pigs euthanized via blunt-force trauma than at all flow rates of 50:50 gas, as well as slow and medium flow rates of CO_2. Out of view and "other" behaviors were observed for less than 0.1% of time for any individual pig.

Neonate pigs

When pigs were conscious, duration of standing and locomotion did not ($P = 0.11$) differ between gas types or flow rates. Duration of standing and locomotion was longer ($P < 0.05$) in AMB compared to all gas and flow rate combinations (Table 4). Duration of oral and nasal behaviors was shorter ($P < 0.05$) in the prefill vs. other flow rates, but was a briefly observed behavior when pigs were exposed to the CO_2 and 50:50 gas types. Duration of licking and chewing was less than 7 s for all neonate pigs, and did not differ between gas types (P = 1.00) or among flow rates (P = 0.88; Table 4).

Within CO_2, prefill elicited open-mouth breathing fastest (P < 0.05) relative to other flow rates (Table 6), and in the fast and medium flow rates open-mouth breathing occurred quicker (P < 0.05) than the slow flow rate. Furthermore, within 50:50, again the prefill treatment elicited open-mouth breathing faster (P < 0.05) than fast and medium. Differences were not observed for latency to open-mouth breathing between the gas types (P = 0.84). Percentages of pigs displaying open-mouth breathing did not (P = 0.81) differ between gas types or flow rates, with 90 to 100% of the pigs displaying this behavior (Table 5). Conversely, only 1 pig displayed open-mouth breathing in the AMB treatment (P < 0.001). The duration of open-mouth breathing was shortest (P < 0.05) in the prefill treatment, regardless of gas type; however, neonates euthanized with 50:50 had greater (P < 0.05) open-mouth breathing durations at the medium than the fast flow rates (Table 4). Moreover, among CO_2-euthanized pigs, open-mouth breathing duration was less (P < 0.05) in those subjected to the fast and medium flow rates than the slow rate.

Latency to loss of posture was faster (P < 0.001) for CO_2 vs. 50:50 at all flow rates (Table 6). Regardless of gas type, prefill was quickest (P < 0.001) to induce loss of posture, whereas latency to loss of posture was greater (P < 0.001) for the slow relative to the other flow rates among the CO_2-euthanized pigs. Conversely, within 50:50, the fast flow rate induced loss of posture faster (P < 0.001) than the medium flow rate.

Prior to loss of posture, 99% of the neonate pigs displayed ataxia. Duration of ataxia was longer (P < 0.05) in 50:50- than CO_2-euthanized pigs at each flow rate (Table 4). Among CO_2-euthanized pigs, the prefill treatment produced the shortest (P < 0.05) duration of ataxia, and the fast and medium flow rates elicited shorter ($P < 0.05$) durations of ataxia than the slow flow rate. Within neonates euthanized with 50:50, duration of ataxia was shortest ($P < 0.05$) in the prefill treatment, and the fast flow rate induced a shorter ($P < 0.05$) ataxia duration than the medium flow rate.

Prior to complete loss of posture, 25 to 65% of pigs displayed a righting response; however, no ($P = 0.24$) differences were observed between gas types or among flow rates for prevalence of this parameter (Table 5). The number of righting attempts by a single neonate pig was less than 6 for both CO_2 euthanasia (maximum righting attempts were 3, 3, 3, 4 for prefill, fast, medium, and slow flow rates, respectively; results not shown) and 50:50 euthanasia (3, 5, 6 for prefill, fast, and medium flow rates, respectively; results not shown). In addition, the prevalence of muscle excitation did not differ ($P = 0.64$) between gas types or among flow rates, though this was a rare event, occurring in 0 to 15% of pigs by treatment (Table 5).

Regardless of gas type or flow rate, 90 to 100% of the neonate pigs displayed gasping behavior, whereas none of the AMB-treated pigs performed this behavior; however, a longer ($P < 0.05$) duration of gasping was observed in 50:50 vs. CO_2 euthanasia, regardless of flow rate. Within CO_2 euthanasia, duration of gasping was longest ($P < 0.05$) for the slow flow rate, and gasping was longer ($P < 0.05$) for the medium flow rate relative to prefill (Table 4); gasping duration was similar for prefill and the fast flow rates. Among 50:50-euthanized pigs, prefill and medium flow rates produced longer ($P < 0.05$) gasping durations that the fast flow rate (Table 4).

Latency to last movement was longer ($P < 0.05$) for neonate pigs euthanized with 50:50 than CO_2, regardless of flow rate (Table 6).Within CO_2 euthanasia, latency to last movement was longest ($P < 0.05$) for the slow flow rate and shortest ($P < 0.05$) in the prefill treatment, whereas, among 50:50-euthanized pigs, latency to last movement was longer ($P < 0.05$) for the medium than the fast flow rate. Furthermore, latency to last movement was longer ($P < 0.05$) for blunt-force trauma than the CO_2 prefill treatment, but was shorter ($P < 0.05$) than the CO_2 slow flow rate and all 50:50 flow rates. Out of view and "other" behaviors were observed for less than 0.1% of time for any individual pig.

Escape attempts were only observed in 5, 5, and 10% of the neonate pigs in prefill CO_2, prefill 50:50, and AMB treatments, respectively (Table 5). In addition, there were no differences among gas types, flow rates, or AMB for the percentage of pigs displaying defecation ($P = 0.44$), urination ($P = 0.83$), salivation ($P = 0.83$), and nasal discharge ($P = 0.31$; Table 5). Blood was never visible in any discharge, and ocular orbit discharge and vomiting were not observed in any euthanized neonate pigs.

Comparison between age groups

Weaned pigs displayed longer ($P < 0.05$) durations of licking and chewing than neonate pigs in the CO_2 slow, CO_2 fast, and the 50:50 fast treatments ($P < 0.05$; Table 4). A greater ($P < 0.05$) latency to open-mouth breathing was observed in weaned pigs relative to neonates at all gas type and flow rate combinations, except CO_2 fast, whereas duration of open-mouth breathing was also longer ($P < 0.05$) in weaned than neonate pigs for both CO_2 and 50:50 prefill, CO_2 medium flow rate, and 50:50 fast flow rate (Table 4). Loss of posture occurred later ($P < 0.05$) for the weaned than neonatal pigs in the CO_2 slow and medium flow rates, as well as in all 50:50 flow rates (Table 6). Furthermore, duration of ataxia was shorter ($P < 0.05$) for weaned than neonate pigs, especially when euthanized in the CO_2 fast and 50:50 medium treatments (Table 4). Weaned pigs were more ($P < 0.01$) likely to display muscle excitation relative to neonates for all gas types and flow rates except 50:50 prefill. The latency to last movement was numerically longer for weaned pigs relative to the neonates for all gas types and flow rates except CO_2 fast and AMB, and was longer ($P < 0.05$) in the CO_2 prefill and slow and the 50:50 prefill. Weaned pigs were more ($P < 0.01$) likely to defecate than neonate pigs (Table 5), and more ($P < 0.05$) likely to display nasal discharge than neonates when euthanized in CO_2 at the slow and medium flow rates (Table 5).

DISCUSSION

Results from the current study indicated that 100% CO_2 gas, rather than 50:50 CO_2:Ar gas mixture, and fast, rather than slow, flow rates were advantageous for pig welfare and efficacy when euthanizing both weaned and neonate pigs. In this study, the euthanasia process was separated into 2 phases, conscious and unconscious. In the present study, the transition from conscious to unconscious was determined by loss of posture, which has been identified in previous research as an indicator for loss of consciousness (Forslid, 1987; Raj and Gregory, 1996; Velarde et al., 2007). However, there is a transition phase prior to loss of posture during which a number of behaviors are typically observed, including open-mouth breathing, ataxia, and righting response. The level of awareness, hence capacity of animals to suffer, during this transition is unclear; to ensure pig welfare, a conservative approach was taken, with inclusion and assessment of behaviors during this transition. As behavioral responses were the primary measurement, it was important to allow the pigs to display a full and more natural repertoire of behaviors than can be achieved with more invasive methods requiring restraint.

Distress associated with gas euthanasia likely includes fear responses associated with placement in a novel environment and separation from the group in addition to direct responses to the gases. In an attempt to minimize the effects of novelty and social isolation, pigs were provided 10 min exposure to the box before euthanasia and tested in pairs. Whether these attempts were successful is unknown because there were no control groups where pigs were tested singly and without prior exposure to the box.

Behaviors chosen for welfare assessment included those associated with physiological distress, such as open-mouth breathing (Forslid, 1987; Martoft et al., 2002; Mota-Rojas et al., 2012), or psychological distress, such as escape (Blackshaw et al., 1988; Velarde et al., 2007), righting response (Grandin, 1998; Kohler et al., 1999; AVMA, 2013), defecation, and urination. Once unconscious, the point of interest shifted from welfare to efficacy; it is vital that the process be practical for on-farm implementation. This experiment is the first to describe the duration of exposure at different flow rates required for reliable euthanasia of nursery age (weaned) and suckling pigs (neonate). These parameters are important in identifying when the process is not occurring within acceptable guidelines, indicating intervention is necessary. For the purpose of this study, last movement was the best indicator of death because respiratory arrest (the cessation of gasping) was the last movement observed in gas treatments. For pig welfare and practical reasons on-farm, it is critical to reduce the number of pigs that require a secondary euthanasia step.

Gas Type

Pig welfare was superior with 100% CO_2 when compared to 50:50 based on a reduction in the duration of open-mouth breathing, duration of ataxia, prevalence of escape attempts, and righting response duration and intensity. None of the parameters measured indicated superior welfare with the use of 50:50.

At 10% CO_2 concentrations, the majority of human subjects report experiencing breathlessness (described as being unpleasant), and 50% CO_2 concentration is reported as being very pungent (Gregory et al., 1990). There is evidence of capacity for direct sensation and perception of CO_2 gas though the trigeminal nerve in rats (Anton et al., 1991), humans (Anton et al., 1992), and chickens (McKeegan et al., 2005), but this has not been examined in pigs. Open-mouth breathing is a physiological reaction associated with dyspnea (Burki and Lee, 2010), and has been identified as an indicator of compromised welfare in the pig (Velarde et al., 2007). It is important to note other researchers have used hyperventilating (Martoft et al., 2002), respiratory distress (Raj and Gregory, 1996) and gasping (Rodríguez et al., 2008) when describing this behavior. While the onset of this behavior is noted by several researchers (Forslid, 1987; Raj and Gregory, 1996; Martoft et al., 2002), none reported duration of open-mouth breathing. Using onset of open-mouth breathing until onset of loss of posture, the duration of open-mouth breathing can be calculated for some previous research, and values were similar to the current study for the CO_2 prefill treatment (12 and 15 s for 90% CO_2 in Raj and Gregory (1996) and Rodríguez et al. (2008), respectively). It can be argued open-mouth breathing duration is an important measure of distress, and the 50:50 treatments resulted in 60 to 90% longer duration of open-mouth breathing in weaned pigs.

Ataxia is likely an indicator of impaired function of the cerebellum; however, it is unclear how this correlates to impaired cortical function. If ataxia indicates that the pig is aware of its surroundings but is unable to react in a coordinated manner, this could be considered distressing to the pig. In this study, ataxia was defined as a potential stressor for the pig, and, therefore, a shorter duration of ataxia would be associated with improved welfare. Duration of ataxia was twice as long for weaned pigs with 50:50 relative to CO_2 at prefill and fast flow rates. Even though latency to ataxia was discussed by Raj and Gregory (1995) and Troeger and Woltersdorf (1991), the duration of ataxia was not examined until the present experiment.

Escape attempts have been noted by several researchers to be an indicator of compromised welfare and, as such, the goal is to reduce its prevalence (Blackshaw et al., 1988; Kirkden and Pajor, 2006; Velarde et al., 2007). Escape attempts in this study were rare in comparison to other studies (Raj and Gregory, 1996; Velarde et al., 2007). A maximum of 15% of weaned pigs in a particular treatment displayed escape attempts, which only occurred when euthanized with 50:50. Similarly, Raj and Gregory (1996) did not observe escape attempts when pigs were exposed to 80 or 90% CO_2, which they attributed to the pigs not having time to display the behavior. In the current study, pigs were placed in ambient air before the atmosphere was modified for all but the prefill treatments. Thus, there was adequate time for pigs to display escape behavior, as demonstrated in 50:50 treatments. Velarde et al. (2007) observed 33 to 93% of grower pigs displaying escape; however, this was likely due to a more liberal definition that included pigs running across the dip-lift. Only neonate piglets attempted escape when exposed to ambient air. Escape was observed by Raj and Gregory (1996) when grower pigs were individually exposed to AMB, which they attributed to isolation and caging distress. Because pigs in the current study were placed in the box with a conspecific, the novel environment and separation from the dam were more likely causes of this behavior in AMB. Separation from the dam may also explain why escape attempts in AMB were only seen in the neonates and not the weaned pigs.

The lack of a righting reflex has been cited to be critical to ensure the pig has been successfully rendered unconscious prior to slaughter (Sandström, 2009; Grandin, 2010), and is cited as an indicator of unconsciousness (Anil, 1991; NPB, 2009). However, duration and frequency of righting responses have not been quantified within an individual pig as a measure of distress. In the current study, the righting responses scored appeared to be coordinated but failed attempts to right, not a simple reflex response. Righting responses require coordinated brain activity, and are indicators of brain function and not a simple reflex response. Because CO_2 and Ar are both heavier than air, it is possible that some of the observed righting responses reflect the pig's attempt to physically avoid the gas; therefore, duration and intensity (frequency) of righting responses were used as indicators of distress in this study. In the weaned pig, righting response duration was 9-fold greater and displayed by twice as many 50:50-euthanized pigs compared to pigs euthanized with CO_2, in the prefill treatment.

Latency to loss of posture was greater for 50:50 at most flow rates, which is in sharp contrast to Raj (1999), who found latency to loss of posture was not affected by gas type when finisher pigs were exposed to 90% Ar, 80 to 90% CO_2, or 30:60 CO_2:Ar mixture. Additionally, the latencies to loss of posture (15 to 18 s) reported by Raj (1999) were considerably shorter than observed in the current study, perhaps due to differences in age and weight. It is important to note that Ar is a noble gas with no known effect on the body, and likely causes unconsciousness through hypoxia. Therefore, it was surprising that 90% Ar was capable of producing loss of posture in less than 20 s (Raj, 1999) when compared to 103 s and 45 s observed in weaned and neonate pigs euthanized using the 50:50 prefill treatment, respectively, in this study. Another factor may be the method of gas application. When using CO_2 to stun prior to slaughter, pigs are lowered into a pit where a constant modified atmosphere is present. Yet, in the current experiment, the prefilled box allowed some reintroduction of atmospheric air when the lid was opened to place the pigs inside, whereas gas flow was initiated after the pigs were placed in the box in other treatments. Both of these methods produced different exposure when compared to the slaughter conditions used by

Raj (1999). The current study supports the findings of Meyer et al. (2013), who demonstrated a 30:70 CO_2:N gas mixture provided at 20% box volume/min caused an increased latency to loss of righting response relative to 100% CO_2.

For both the weaned and neonate pigs, greater latency to death, as determined by last movement, was observed for 50:50 at all flow rates. In the weaned pig, latency to last movement was 1.7 times greater for prefill 50:50 than CO_2. More importantly, 50:50 at the slow flow rate had an efficacy rate of only 15% within the parameters of this experiment (10 min allowed for loss of consciousness and 10 min allowed for death post-loss of consciousness), which is unacceptable for both ethical and practical reasons. However, all other flow rate and gas type combinations were 100% successful. Dykshorn and Donovan (2010) found 100% CO_2 to be 83.9 to 97.7% effective, depending on the duration of exposure time. However, flow rate details were not provided by these authors making a direct comparison difficult.

Flow Rate

Pig welfare was superior with faster flow rates, being associated with lower duration and intensity of behavioral indicators of distress, as well as decreased latency of indicators of efficacy (loss of posture, gasping, and last movement). Among CO_2-euthanized pigs, the slow flow rate more than doubled the duration of open-mouth breathing, ataxia, and righting response compared to prefill. Additionally, the slow flow rate resulted in a 5-fold increase in latency to loss of consciousness (loss of posture) and 2-fold increase in latency to death (last movement). These results are quite similar to those of Sutherland (2010), who examined effects of prefill and slow flow rates with 90% CO_2 on latency to loss of brain activity and heart rate. However, the current findings conflict with recommendations for rodents reported from the Newcastle Census Meeting (Hawkins et al., 2006), which concluded a 20% flow rate was preferred over prefilled, based on many factors (with heavy emphasis on the human experience), such as low CO_2 concentrations causing aversion due to dyspnea vs. concentrations above 50% causing pain. Subsequent rodent research indicated that aversion occurs even at lower gas concentrations. In rats, Niel et al. (2008) examined 100% CO_2 with flow rates from 3 to 27% (box volume exchange rate/min) where rats were trained to enter the box for a food reward and allowed to exit at will. Minimal response to flow rates was observed, with rats leaving when CO_2 concentrations reached 11 to 16% (long before loss of consciousness), and all rats exited the box before loss of consciousness. In a similarly designed study, Makowska et al. (2008) examined 100% Ar with flow rates from 40 to 239% (box exchange rate/min), and, again, minimal response to flow rate was observed, with rats leaving when O_2 concentrations reached 6 to 9%, and well before loss of consciousness (all rats exited the box prior to loss of consciousness). These results suggest that both hypercapnia and hypoxia are inherently aversive even at low levels, and call into question prolonged gas exposure for euthanasia. Based on the parameters measured in the present study and other studies involving swine and rats, slow flow rates prolong the duration of the process, and, more importantly, suffering, without providing benefits to animal welfare. However, preference testing is a more robust research tool for determining the subjective experience of pigs and relative aversion to different flow rates and still needs to be explored in the pig.

Age

It has been demonstrated in several species that achieving successful euthanasia for neonates may take longer or require a higher gas concentration relative to the more mature animal (AVMA, 2013). In addition, anecdotal reports from stockpersons indicated a belief that neonates are more difficult to euthanize than older pigs. This research indicated the opposite effect, because neonate pigs succumbed to the gases faster than weaned pigs for both the conscious (loss of posture) and unconscious (last movement) phases. Additionally, during the conscious phase, signs of distress were less for neonates relative to weaned pigs as measured by defecation, nasal discharge, and duration of open-mouth breathing. Duration of ataxia was the single parameter for which neonate pigs displayed greater distress when compared to weaned pigs. Although Sutherland (2010) observed small but statistically significant differences for pigs aged 1, 2, 3, 4, and 5 and 6 wk of age, she concluded that these small differences did not merit development of different euthanasia methodologies for pigs of different ages, which is supported by the current findings.

SUMMARY

When examining euthanasia and welfare culling (Whiting and Marion, 2011) methods, both animal welfare and efficacy are key components. Welfare is composed of both duration and intensity of distress during the conscious phase, and the results from this study indicate that pigs succumb faster when using 100% CO_2 compared to a 50:50 CO_2:Ar gas mixture. Differences were not observed between gases or flow rates for many of the welfare parameters measured; however, when differences were observed, 100% CO_2 resulted in shorter durations of behavioral indicators of distress and physiological responses. Thus, proposed benefits of adding Ar were not observed. Likewise, the slow flow rate increased the durations of sensation and distress measures, while resulting in longer latencies to loss of posture and last movement. The current study was able to conclude that 50:50 CO_2:Ar gas mixtures and slower flow rates should be avoided when euthanizing weaned and neonate pigs. Many farms are using a 2- or 3-min gas run time, followed by a 5-min dwell time, or a similarly timed procedure. Such recommendations should only be made based on the underlying principles of gas displacement, not simply time alone. It is important to note that if a procedure similar to slow flow in this trial had been followed on farm, most pigs would not have been successfully euthanized. It is critical that farms know the flow rate of their systems and avoid designing euthanasia procedures solely on timing.

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Table 1. Temperature and relative humidity during euthanasia of weaned pigs

Treatments[1]	Temperature, °C Starting[2]	Temperature, °C Change[3]	Relative humidity, % Starting[2]	Relative humidity, % Change[3]
AMB	26.3	-0.3	62.4	2.0
CO_2				
Prefill	26.4	-0.1	62.3	2.1
Fast	26.3	-0.3	73.6	5.4
Medium	25.8	-0.2	68.5	4.7
Slow	26.1	-0.0	64.4	3.9
50:50				
Prefill	26.3	-0.4	70.6	2.5
Fast	26.2	-0.2	72.3	3.7
Medium	26.2	-0.2	71.6	4.5
Slow	26.5	-0.1	63.4	3.8
SEM	0.8	0.1	5.0	2.8

[1]AMB = pigs euthanized by blunt-force trauma after being placed in the box for 10 min; CO_2 = euthanized with 100% CO_2; 50:50 = euthanized with a 50:50 mixture of CO_2 and argon; Prefill = pigs placed in box prefilled with the appropriate gas followed by a gas flow rate of 20% box volume/min; Fast = gas flow rate of 50% box volume/min; Medium = gas flow rate of 35% box volume/min; and Slow = gas flow rate of 20% box volume/min.

[2]Recorded upon pig placement in euthanizing box.

[3]Change that occurred in the box from the time of placement until pig removal from the box.

Table 2. Temperature and relative humidity during euthanasia of neonate pigs

	Temperature, °C		Relative humidity, %	
Treatments[1]	Starting[2]	Change[3]	Starting[2]	Change[3]
AMB	23.1	0.0	79.2	9.2
CO_2				
Prefill	22.6	-0.0	55.4	1.5
Fast	23.6	-0.0	46.0	2.6
Medium	23.5	-0.1	57.4	4.6
Slow	23.3	-0.0	61.2	4.3
50:50				
Prefill	22.7	-0.4	55.4	2.9
Fast	22.8	-0.2	52.9	3.7
Medium	23.3	-0.1	59.4	4.5
Slow	---	---	---	---
SEM	0.8	0.1	3.9	2.8

[1]AMB = pigs euthanized by blunt-force trauma after being placed in the box for 10 min; CO_2 = euthanized with 100% CO_2; 50:50 = euthanized with a 50:50 mixture of CO_2 and argon; Prefill = pigs placed in box prefilled with the appropriate gas followed by a gas flow rate of 20% box volume/min; Fast = gas flow rate of 50% box volume/min; Medium = gas flow rate of 35% box volume/min; and Slow = gas flow rate of 20% box volume/min.

[2]Recorded upon pig placement in euthanizing box.

[3]Change that occurred in the box from the time of placement until pig removal from the box.

Table 3. Ethogram developed for investigating latency, duration, prevalence, and frequency of behavioral indicators of distress and insensibility during euthanasia.

Postures	Definition	Direct	Video[1]
Standing/ Locomotion[2]	Maintaining an upright and stationary body position by supporting the BW on the feed with the legs extended or movement derived from the repulsive force from the action of the legs.		
Sitting[2]	A body position in which the posterior of the body trunk is in contact with the ground, sides of the box, or the other pig, and supports most of the BW.		
Lying[2]	Maintenance of a recumbent position.		
Ataxic movement[3]	Pig is moving in a seemingly uncoordinated fashion; lack of muscle coordination during voluntary movement.		
Righting response	Pig is making attempt to maintain either a standing or lying sternal posture but is not successful in maintaining the position (different from muscular excitation in that these are slower and seemingly coordinated movements); The event was defined as each time effort was made and the muscle relaxed.		
Muscular excitation[4, 5]	Repeated muscular movement of the whole body, including head movements upward; seemingly uncoordinated (categorizing posture is not possible due to rapid and frequent movements); severe excitation appears as major clonic convulsive seizures.		
Out of view	Pig could not be seen clearly enough to identify the behavior or position, or pig was removed from box.		
Other	Pig's posture was not defined by previous definitions.		
Behaviors			
Oral Nasal Facial (ONF)[6]	Rubbing, licking, biting, touching the mouth, snout or face to 1 of 2 modifiers – other pig or item (walls, flooring, or cage).	*	
Licking and chewing[6]	Pig is going through motions of licking and chewing, similar to ONF but not interacting with an object or the other conspecific.	*	
Open-mouth breathing[7, 8]	Pig's mouth is open, taking in quick breaths (panting), with distinct thoracic movements; upper and lower jaw being held open with the top lip pulled		

	back, exposing gums or teeth and panting (pronounced inhalation and exhalation observed at the flanks).	
Gasping[4, 7]	Rhythmic breaths characterized by very prominent and deep thoracic movements, with long latency between (may involve stretching of the neck); often occurs right before or after loss of posture.	*
Out of view	Pig could not be seen clearly enough to identify the behavior or position, or pig was removed from box.	
Other	Pig's behavior was not defined by previous definitions.	
Events		
Salivation	Fluid discharge coming from mouth; type of discharge (may be clear and fluid, viscous, or blood) was noted.	
Nasal Discharge	Discharge from the nasal cavity; type of discharge (may be clear and fluid, viscous, or blood) was noted.	
Eye orbit discharge	Discharge from the ocular orbit; type of discharge (may be clear and fluid, viscous, or blood) was noted.	
Defecation[2]	Elimination of feces from the body.	
Urination[2]	Discharge of urine from the body.	
Vomiting[2]	Ejection of gastrointestinal contents through the mouth.	
Escape attempt, bout[7,9]	Pig is raising their forelegs on the side of the wall of the box or pushing quickly and forcefully with their head or nose on the lid of the box; forceful coordinated movement against the exterior of the box; occurrences within a 10-s period will be recorded as a single bout.	
Loss of posture[7,9]	Pig is slumped sown, making no attempt to right itself; may follow a period of attempts to maintain posture (considered first indicator of loss of consciousness).	
Last Movement	No movement, of any kind is observed from the pig.	

*All direct observations were scored as events

[1]For video, each pig was scored for 1 of 8 mutually exclusive postures and complementary for 1 of 6 mutually exclusive behaviors, along with event behaviors when occurred.

[2]Adapated from Hurnik et al. (1985).

[3]Adapted from Blood et al. (2007).

[4]Adapted from Dodman (1977).
[5]Adapated from Rodríguez et al. (2008).

[6]Adapated from Meiszberg et al. (2009).
[7]Adapted from Velarde et al. (2007).
[8]Adapted from Johnson et al. (2010).
[9]Adapted from Raj and Gregory (1996).

Table 4. Effect of euthanasia method on the mean duration ± SE (seconds) of weaned neonate pigs displayed behavioral indicators of distress

		100% CO_2 gas[1]				50:50 CO_2 & argon gas mixture (50:50)[1]			
Distress measures[2], s	AMB[3]	Prefill	Fast	Medium	Slow	Prefill	Fast	Medium	Slow
				Weaned pigs					
Standing and locomotion	241 ± 18[a]	15 ± 30[b]	58 ± 25[b]	61 ± 15[b]	79 ± 15[b]	23 ± 31[b]	76 ± 26[b]	86 ± 15[b]	115 ± 15[b]
Oral nasal, all	139.3 ± 5.0[a]	0.0 ± 0.0[c]	3.6 ± 1.3[b]	3.6 ± 1.0[b]	5.2 ± 1.3[b]	0.0 ± 0.0[c]	5.1 ± 1.3[b]	4.1 ± 1.5[b]	11.1 ± 3.6[b]
Licking and chewing	27.3 ± 5.0[a]	1.4 ± 0.4[b]	20.7 ± 2.5[a]	11.0 ± 1.7[a]	18.4 ± 2.7[a]	4.0 ± 1.2[b]	20.2 ± 2.9[a]	13.1 ± 2.9[a]	33.6 ± 7.5[a]
Open-mouth breathing	0[f]	20 ± 2[e]	26 ± 2[d]	34 ± 2[c]	45 ± 3[b]	35 ± 2[c]	46 ± 3[b]	64 ± 5[a]	72 ± 5[a]
Ataxia	0[g]	14 ± 1[f]	19 ± 2[e]	21 ± 2[d]	39 ± 4[c]	35 ± 5[c]	40 ± 5[c]	46 ± 5[b]	52 ± 5[a]
Righting response	0[d]	1.2 ± 0.7[c]	0.3 ± 0.8[c]	3.7 ± 1.7[b]	4.2 ± 1.3[b]	11.2 ± 2.6[a]	8.7 ± 2.2[a,b]	4.7 ± 2.2[b]	13.7 ± 3.2[a]
Clonic movement	0[d]	50 ± 7[c]	55 ± 5[b,c]	61 ± 5[b,c]	84 ± 6[a]	79 ± 6[a,b]	65 ± 5[b,c]	70 ± 5[b,c]	93 ± 6[a]
Gasping	0[e]	224 ± 12[c]	174 ± 11[d]	198 ± 12[c,d]	346 ± 12[b]	371 ± 13[b]	280 ± 12[c]	344 ± 12[b]	478 ± 12[a]
				Neonate pigs					
Standing and locomotion	309 ± 18[a]	15 ± 30[b]	36 ± 15[b]	37 ± 15[b]	55 ± 15[b]	8 ± 31[b]	48 ± 26[b]	58 ± 15[b]	---
Oral nasal, all	53.3 ± 21.6[a]	0.0 ± 0.0[c]	1.4 ± 1.3[b]	1.0 ± 1.0[b]	0.7 ± 1.3[b]	0.0 ± 0.0[c]	2.0 ± 1.3[b]	3.4 ± 1.5[b]	---
Licking and chewing	6.9 ± 4.8	0.0 ± 0.0	5.3 ± 2.5	4.0 ± 1.7	5.2 ± 2.7	1.3 ± 2.0	2.9 ± 3.0	2.0 ± 3.0	---
Open-mouth breathing	0[e]	12 ± 2[d]	23 ± 2[c]	24 ± 2[c]	39 ± 3[b]	14 ± 2[d]	34 ± 3[b]	49 ± 5[a]	---
Ataxia	0[e]	14 ± 1[d]	27 ± 2[c]	26 ± 2[c]	43 ± 4[b]	24 ± 5[c]	47 ± 5[b]	66 ± 5[a]	---
Righting response	0[b]	2.3 ± 0.7[a]	3.6 ± 0.8[a]	4.9 ± 1.7[a]	1.9 ± 1.3[a]	3.7 ± 2.6[a]	4.6 ± 2.2[a]	8.0 ± 2.2[a]	---
Clonic movement	0[d]	41 ± 5[b,c]	37 ± 5[b,c]	27 ± 5[c]	33 ± 6[c]	66 ± 6[a]	59 ± 5[a]	58 ± 6[a,b]	---
Gasping	0[f]	210 ± 12[e]	225 ± 11[e]	247 ± 12[d,e]	348 ± 12[b]	374 ± 12[a]	308 ± 13[c]	346 ± 12[b]	---

[a – g]Within a row, lease squares means (± SE) lacking a common superscript letter differ, $P < 0.05$.

[1]Prefill = pigs placed in box prefilled with the appropriate gas followed by a gas flow of 20% box volume/min; Fast = gas flow rate of 50% box volume/min; Medium = gas flow rate of 35% box volume/min; and Slow = gas flow rate of 20% box volume/min.

[2]Refer to Table 3 for description of each behavior.

[3]AMB = pigs euthanized by blunt-force trauma after being placed in the box for 10 min.

Table 5. Effect of euthanasia method on the frequency (%) of weaned and neonate pigs displaying behavior indicators of sensation and distress

		100% CO_2 gas[1]				50:50 CO_2 & argon gas mixture (50:50)			
Distress measures[2], %	AMB[3]	Prefill	Fast	Medium	Slow	Prefill	Fast	Medium	Slow
	--------Weaned pigs--------								
Standing and locomotion	100	100	100	100	100	100	100	100	100
Oral nasal, all	90	0	40	45	65	0	55	40	55
Licking and chewing	40	5	70	60	45	10	50	40	44
Escape attempts	0[b]	0[b]	0[b]	0[b]	0[b]	15[a]	10[a]	15[a]	15[a]
Defecation	35	25	45	45	50	50	60	50	45
Urination	10	15	20	10	10	30	35	35	5
Salivation	15	5	5	0	15	10	10	30	50
Nasal discharge	10	0	10	20	25	15	5	20	30
Open-mouth breathing	0	80	100	100	100	100	90	90	100
Ataxia	0	10	25	35	20	60	55	60	55
Righting response	0	20	10	25	35	55	60	55	60
Muscle excitation	0[d]	65[a]	60[a]	60[a]	40[b]	30[c]	45[b]	40[b]	20[c]
Clonic movement	0	95	100	100	100	100	100	100	100
Gasping	0	90	100	100	100	100	100	90	95
	--------Neonate pigs--------								
Standing and locomotion	100	100	100	100	100	100	100	100	---
Oral nasal, all	90	0	15	25	15	55	45	35	---
Licking and chewing	5	0	30	30	20	25	40	30	---
Escape attempts	10	5	0	0	0	5	0	0	---
Defecation	30	20	25	25	10	20	30	30	---
Urination	20	20	35	25	20	15	30	30	---
Salivation	5	5	10	5	10	5	5	5	---
Nasal discharge	5	0	0	5	5	0	0	15	---
Open-mouth breathing	5	90	90	100	100	100	100	100	---
Ataxia	0	95	100	100	100	100	100	100	---

Righting response	0	50	50	60	25	65	45	60	---
Muscle excitation	0	10	5	0	0	15	0	0	---
Clonic movement	0	90	100	85	85	100	100	95	---
Gasping	0	90	100	100	100	100	100	90	---

[a-d]Within a row, prevalence of pigs (%) lacking a common superscript letter differ, $P < 0.05$.

[1]Prefill = pigs placed in box prefilled with the appropriate gas followed by a gas flow of 20% box volume/min; Fast = gas flow rate of 50% box volume/min; Medium = gas flow rate of 35% box volume/min; and Slow = gas flow rate of 20% box volume/min.

[2]Refer to Table 3 for description of each behavior.

[3]AMB = pigs euthanized by blunt-force trauma after being placed in the box for 10 min.

Table 6. Effect of euthanasia method on the mean latency ± SE (seconds) of weaned neonate pigs displayed behavioral indicators of distress

Distress measures[2], s	AMB[3]	100% CO_2 gas[1]				50:50 CO_2 & argon gas mixture (50:50)[1]				
		Prefill	Fast	Medium	Slow	Prefill	Fast	Medium	Slow	SEM
		Weaned pigs								
Open-mouth breathing	---	11[d]	55[c]	59[c]	87[b]	28[d]	65[c]	86[b]	113[a]	33
Loss of posture	---	35[e]	89[d]	102[d]	143[c]	90[d]	148[c]	174[b]	238[a]	7
Last movement	221[e]	269[e]	274[e]	313[b]	529[b]	451[c]	297[d]	467[b]	775[a]*	40
		Neonate pigs								
Open-mouth breathing	---	7[c]	45[b]	54[b]	67[a]	12[c]	55[b]	61[b]	X	33

[a - e]Within a row, lease squares means (± SE) lacking a common superscript letter differ, $P < 0.05$.

[1]Prefill = pigs placed in box prefilled with the appropriate gas followed by a gas flow of 20% box volume/min; Fast = gas flow rate of 50% box volume/min; Medium = gas flow rate of 35% box volume/min; and Slow = gas flow rate of 20% box volume/min.

[2]Refer to Table 3 for description of each behavior.

[3]AMB = pigs euthanized by blunt-force trauma after being placed in the box for 10 min.

*If pigs were insensible, they were allowed to remain in the box for up to an additional 600 s